

Short Communication

Effects of Electron-Beam Irradiation on Whole Genome Amplification

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Abstract

Electron-beam (E-beam) irradiation, currently being used to sterilize mail addressed to selected ZIP codes in the United States, has significant negative effects on the genomic integrity of DNA extracted from buccal-cell washes. We investigated the yield, composition, and genotyping performance of whole genome amplified DNA (wgaDNA) derived from 24 matched samples of E-beam-irradiated and nonirradiated genomic DNA (gDNA) as a model for the effects of degraded gDNA on the performance of whole genome amplification. gDNA was amplified using the Multiple Displacement Amplification method. Three methods of DNA quantification analysis were used to estimate the yield and composition of wgaDNA, and 65 short tandem repeat and single nucleotide polymorphism genotyping assays

were used to evaluate the genotyping performance of irradiated and nonirradiated gDNA and wgaDNA. Compared with wgaDNA derived from nonirradiated gDNA, wgaDNA derived from irradiated gDNA exhibited a significantly reduced yield of wgaDNA and significantly reduced short tandem repeat and single nucleotide polymorphism genotyping completion and concordance rates ($P < 0.0001$). Increasing the amount of irradiated gDNA input into whole genome amplification improved genotyping performance of wgaDNA but not the level of wgaDNA derived from nonirradiated gDNA. Multiple Displacement Amplification wgaDNA derived from E-beam-irradiated gDNA is not suitable for genotyping analysis. (Cancer Epidemiol Biomarkers Prev 2005;14(4):1-4)

In response to the October 2001 anthrax attacks in the United States, the U.S. Postal Service initiated a mail sterilization program for mail addressed to government offices within the 202 through 205 ZIP codes utilizing electron-beam (E-beam) irradiation as the source of high-energy radiation (1). E-beam irradiation of organic materials leads to the production of secondary species created by absorption of energy from the high-energy particle along ionization tracks (2). The secondary low-energy species are primarily low-energy electrons, which damage DNA by producing single-stranded and double-stranded breaks by both ionization and by molecular resonance mechanisms (3). Whereas the sterilizing properties of high-energy irradiation are well known, the effects of E-beam irradiation on DNA extraction yields and genotyping performance from biospecimens subjected to the U.S. Postal Service irradiation protocol were unknown. Castle et al. (4) evaluated and recently reported significantly reduced yields of high-molecular weight genomic DNA (gDNA) extracted from E-beam irradiated mouthwash specimens and significantly reduced formation of a 989-bp PCR amplicon from the irradiated extracted DNA. To simulate the U.S. Postal Service

irradiation protocol of ≥ 56 kGy of E-beam irradiation, Castle et al. (4) had the mouthwash samples irradiated twice with 10-MeV irradiation (Titan Scan Technologies, San Diego, CA), for a total dose of between 70 and 97 kGy.

Whole genome amplification (WGA) promises to become a widely used method to generate DNA from clinical and epidemiologic specimens for large-scale genotyping (5), especially for samples with limited quantities of gDNA available, such as those collected in population-based epidemiologic studies (6). However, the performance of WGA on irradiated gDNA irradiated by E-beam irradiation has not been evaluated. The Multiple Displacement Amplification (MDA) method of WGA requires an initial gDNA template strand long enough to serve as a hybridization target for multiple (three or more) random oligonucleotide primers on each strand; the optimal minimal size of gDNA templates is related to the processivity of the DNA polymerase being used and the concentration of the primers (7). Recommendations for the amount of gDNA template to be used in the MDA-based WGA reaction are 10 to 100 ng of nondegraded gDNA (5). The reduction of intensity of the largest PCR product tested (989 bp) and the disappearance of high-molecular weight (≥ 23 kbp) gDNA observed in the Castle et al. (4) study suggest that E-beam irradiation may render gDNA suboptimal for MDA WGA. Therefore, we did MDA WGA on the same irradiated and nonirradiated gDNA samples previously analyzed by Castle et al. (4) to evaluate the performance of WGA on gDNA samples exposed to E-beam irradiation and the genotyping performance of the derived whole genome amplified DNA (wgaDNA).

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Materials and Methods

Biospecimens. Mouthwash samples were originally collected in accordance with a National Cancer Institute Institutional Review Board–approved protocol (6). An aliquot of mouthwash specimen was subjected to 10-MeV E-beam irradiation (Titan Scan Technologies) as described (4). The identity of the samples with respect to irradiation status was unknown to the individuals handling the DNA samples and analyzing the resulting yield and genotyping data until all data were collected and placed in analytic data files (SAS, Cary, NC).

Whole Genome Amplification and Yield and Composition of Whole Genome Amplified DNA. For the GenomiPhi DNA Amplification kit WGA protocol (Amersham Biosciences, Piscataway, NJ), 10, 25, 50, and 100 ng of 24 pairs of gDNA samples (E-beam irradiated and nonirradiated) were used as template (input). wgaDNA was quantified using the PicoGreenI assay, using optical densitometry at A_{260} , and using a reverse transcription-PCR assay, as described (8). The mass of ssDNA and dsDNA in each wgaDNA sample was estimated according to the following transformation from the observed PicoGreen and A_{260} measurements: the expected A_{260} measurement for the dsDNA concentration estimated by the observed PicoGreen measurement was calculated assuming 50 ng dsDNA/ $\mu\text{L}/A_{260}$, the calculated expected A_{260} measurement for the dsDNA concentration was subtracted from the observed A_{260} measurement, and the difference was used to calculate the expected ssDNA concentration in the sample assuming 33 ng ssDNA/ $\mu\text{L}/A_{260}$. Total wgaDNA mass was obtained from the sum of the estimated ssDNA and dsDNA masses. Wilcoxon’s rank sum test and Wilcoxon signed rank test for paired samples were used to evaluate differences in yield distributions.

Genotype Analysis. For AmpFISTR Identifier assay genotyping (Applied Biosystems, Inc., Foster City, CA), 2.5 ng of dsDNA (for both gDNA and wgaDNA as determined by PicoGreen) were used as template DNA as previously described (4), whereas 5 ng of dsDNA were used as

template for 49 TaqMan single nucleotide polymorphism (SNP) genotyping assays, as described on the <http://SNP500Cancer.nci.nih.gov> website (9). All DNA samples were genotyped in duplicate and genotype determination of each replicate was independently determined. Scoring of short tandem repeat (STR) alleles was automatically determined using ABI Prism GeneMapper v3.0 software (Applied Biosystems) and SNP genotype clusters were manually scored using Sequence Detection Software 2.0 (Applied Biosystems, Foster City, CA). STR genotype completion, no amplification, and discordance (wgaDNA to gDNA) rates were calculated with a minimum signal strength threshold of 50 relative fluorescence units. SNP genotype completion, undetermined genotype, no amplification, and discordance (wgaDNA to gDNA) rates were calculated. Differences in STR and SNP completion, undetermined genotype, no amplification, and discordance rates were evaluated using contingency table (χ^2 and Fisher exact test and McNemar test for paired samples) significance testing.

Results and Discussion

Yields of wgaDNA were evaluated on 24 matched pairs of wgaDNA derived from irradiated and nonirradiated gDNA, and genotyping performance was evaluated on 48 matched pairs of irradiated and nonirradiated gDNA, and wgaDNA derived from irradiated and nonirradiated gDNA. Irradiation resulted in statistically significant decreases in the mean yield of double-stranded wgaDNA ($P < 0.0001$), human-specific wgaDNA ($P \leq 0.0003$), and total wgaDNA ($P \leq 0.003$; Table 1). There was a statistically significant ($P < 0.0001$) trend for increased single-stranded wgaDNA yield with increased irradiated gDNA input into the WGA reaction (Table 1). Note that the estimation of wgaDNA by optical densitometry does not show significant differences in wgaDNA yield in wgaDNA derived from irradiated and nonirradiated gDNA templates when using the traditional assumption of 50 ng dsDNA/ $\mu\text{L}/A_{260}$, whereas the estimated total wgaDNA does,

Table 1. wgaDNA yields (in nanograms) derived from nonirradiated and irradiated gDNA

Quantification	gDNA input (ng)	Nonirradiated		Irradiated		P*
		Mean	SD	Mean	SD	
A†	10	18,407	1,268	18,440	1,807	0.94
	25	19,207	2,406	18,783	2,005	0.49
	50	18,705	1,633	18,613	2,254	0.86
	100	17,559	2,374	18,905	1,966	0.05
P _{trend} ‡		0.04		0.87		
Reverse transcription-PCR§	10	3,905	891	1,028	1,359	<0.0001
	25	4,216	1,035	1,359	1,493	<0.0001
	50	3,915	1,610	1,494	2,249	0.0003
	100	4,557	1,477	1,337	2,010	<0.0001
P _{trend} ¶		0.26		0.84		
% ssDNA	10	47	3	64	3	<0.0001
	25	48	7	67	4	<0.0001
	50	45	5	68	5	<0.0001
	100	44	12	73	5	<0.0001
P _{trend} ¶¶		0.15		<0.0001		
Total DNA*	10	14,818	867	13,838	1,226	0.003
	25	15,346	1,599	13,976	1,373	0.003
	50	15,143	1,098	13,768	1,493	0.0006
	100	14,306	1,636	13,747	1,337	0.2
P _{trend}		0.05		0.94		

*T test of wgaDNA yield derived from nonirradiated versus irradiated gDNA, assuming normality.
†A, yield of wgaDNA assuming 50 ng dsDNA/ $\mu\text{L}/A_{260}$.
‡P_{trend}, P value from ANOVA.
§Reverse transcription-PCR, human-specific yield of wgaDNA (8).
||% ssDNA as estimated in Materials and Methods.
¶¶Total DNA, the sum of ssDNA in nanograms and dsDNA in nanograms as estimated in Materials and Methods.

Table 2. STR and SNP genotyping performance of wgaDNA derived from nonirradiated and irradiated gDNA

gDNA*	n	Nonirradiated					Irradiated				
		Completed	No amp. [†]	Undet. [‡]	GQ < 0.25 [§]	Concord.	Completed	No amp. [†]	Undet. [‡]	GQ < 0.25 [§]	Concord.
STR gDNA (ref)	24	768 (100%)	0	—	0	768 (100%)	768 (100%)	0	—	1	767 (100%)
10		766 (100%)	2	—	0	764 (100%)	494 (64%) [¶]	274	—	198	233 (48%) [¶]
25		768 (100%)	0	—	0	768 (100%)	595 (78%) [¶]	173	—	134	382 (69%) [¶]
50		746 (97%)	22	—	16	720 (97%)	497 (65%) [¶]	271	—	226	258 (54%) [¶]
100		768 (100%)	0	—	0	768 (100%)	611 (80%) [¶]	157	—	129	397 (68%) [¶]
SNP gDNA (ref)	24	2,329 (99%)	0	23	—	2,328 (100%)	2,341 (100%)	1	10	—	2,341 (100%)
10		2,334 (99%)	1	17	—	2,323 (100%)	1,895 (81%) [¶]	209	248	—	1,563 (82%) [¶]
25		2,328 (99%)	0	24	—	2,271 (98%)	2,018 (86%) [¶]	57	277	—	1,766 (88%) [¶]
50		2,343 (100%)	0	9	—	2,333 (100%)	2,017 (86%) [¶]	27	308	—	1,855 (92%) [¶]
100		2,338 (99%)	1	13	—	2,328 (100%)	2,060 (88%) [¶]	9	283	—	1,971 (96%) [¶]

*gDNA input (ng) into the WGA reaction.

[†]No amplification observed (i.e., no STR allele observed above the threshold of 50 relative fluorescence unit, or a TaqMan data point that clusters with the no template control samples).[‡]Undetermined, a TaqMan datapoint that falls outside of the three genotype clusters and the one no amplification cluster.[§]Genotype quality scores, a composite measure of STR genotype quality.^{||}Concordance compared with gDNA.[¶] $P < 0.0001$, completion and concordance rates, wgaDNA derived from irradiated gDNA vs. nonirradiated gDNA, irradiated gDNA, and wgaDNA derived from non-irradiated gDNA.

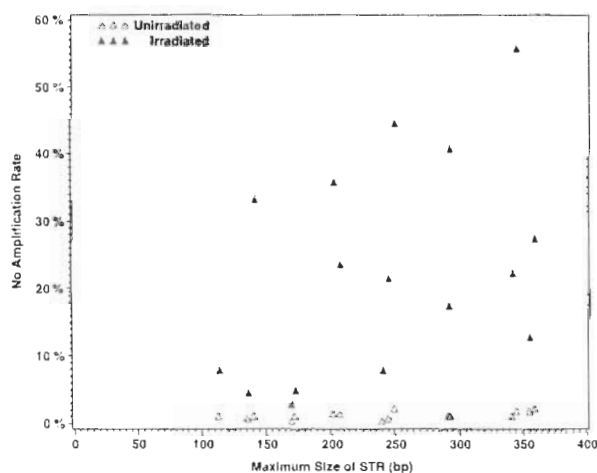
as it accounts for the substantial proportion of ssDNA in the wgaDNA. The absence of a statistically significant increase of human-specific amplifiable DNA (as assessed by reverse transcription-PCR), when increasing the gDNA template input level from 10 to 100 ng, suggests that the increase in the estimated proportion of ssDNA in the wgaDNA derived from irradiated gDNA represents an increase in artifactual wgaDNA, not of human-specific wgaDNA useful for TaqMan genotyping.

In contrast to nonirradiated gDNA, irradiated gDNA, and wgaDNA derived from nonirradiated gDNA, wgaDNA derived from irradiated gDNA exhibited significantly reduced STR and SNP genotyping completion and concordance rates for all gDNA input levels (all $P < 0.0001$; Table 2). Increasing the amount of irradiated gDNA input into the WGA moderated, but did not eliminate, the deleterious effect of irradiation on the STR genotyping performance of wgaDNA derived from gDNA. Specifically, the number of no amplification genotyping failures was reduced, significantly for SNP genotypes (Spearman rank correlation = -1.0 , $p < 0.0001$), but

the number of low-quality STR genotypes (genotype quality score of < 0.25) and the number of undetermined SNP genotypes were not reduced.

Castle et al. (4) observed that irradiation treatment of gDNA resulted in significant reductions in the intensity of larger PCR products when visualized either on agarose gels or using capillary electrophoresis. In addition, using the same STR panel as used in this study, Castle et al. (4) observed a significant effect of STR PCR product size ($P < 0.005$) and a significant interaction between STR PCR product size and irradiation treatment ($P = 0.01$) on STR PCR product failure. In this study, there was a significant overall correlation between STR PCR product size (range, 112-359 bp) and no amplification failure rate (Spearman coefficient = 0.47 ; $P < 0.001$) in irradiated gDNA and wgaDNA samples derived from irradiated gDNA. In linear regression models examining STR no amplification failure and discordance rates versus STR PCR product size and irradiation treatment status, irradiation treatment was significantly related to both no amplification failure and discordance rates ($P < 0.0001$), but STR PCR product size was significantly related only to no amplification failure rate ($P = 0.001$). Similarly, the interaction between STR PCR product maximum size and irradiation treatment was significant only for no amplification failure rate ($P = 0.002$; Fig. 1).

Consistent with previous observations of poor PCR amplification of larger templates from irradiated gDNA (4), we found that wgaDNA derived from irradiated gDNA exhibited decreased genotyping performance, specifically, increased numbers of genotype assay amplification failures and discordant STR and SNP genotypes. These genotyping completion and concordance rates are significantly lower than those observed in MDA wgaDNA derived from suboptimal amounts of undegraded template gDNA and than those observed with optimal amounts of undegraded gDNA as template (10).⁶ The MDA method of WGA requires sufficiently long template DNA to enable multiple primers to anneal, extend, and displace newly extended strands, thereby initiating the multiple displacement mechanism that leads to geometric amplification of template in an isothermal reaction (7). We conclude that E-beam irradiation has negative effects on the

**Figure 1.** STR PCR product maximum size, E-beam irradiation treatment, and no amplification failure rate in wgaDNA derived from irradiated and nonirradiated gDNA.⁶ Authors' unpublished data.

quality of gDNA from mouthwash specimens, which resulted in poor-quality wgaDNA as the likely result of DNA fragmentation and reduced levels of displacement amplification. The effects of irradiation on biospecimen DNA collected using other protocols may differ due to the effects of high-energy irradiation on biospecimen-associated organic or protocol-associated inorganic compounds. Irradiated gDNA may serve as a useful model of degraded gDNA template for the development and optimization of alternative methods of WGA. For example, methods of WGA that employ enzymatic, chemical, or physical degradation of gDNA to reduce DNA complexity before amplification may prove to be more useful for such templates (11, 12). Finally, researchers engaged in molecular epidemiologic studies need to be aware of the effect of irradiation on biospecimen gDNA sent through the U.S. Postal Service mail system. The U.S. Postal Service is engaged in a multiyear assessment and implementation of various technologies to reduce the risk of biohazards, and the irradiation approach to sterilizing the mail is a prominent, if not widespread, feature of existing and planned operations, although major private sector couriers have not announced any such plans for their operations.

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